

## **Feeding Behavior and Survival of Glucose-Averse *Blattella germanica* (Orthoptera: Blattoidea: Blattellidae) Provided Glucose as a Sole Food Source**

Jules Silverman<sup>1,2</sup> and Hanneliese Selbach<sup>1</sup>

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Glucose-averse (Glu/Glu) male German cockroaches, *Blattella germanica*, rejected brief exposures to glucose solutions despite food deprivation so extreme as to cause high mortality. In the 24-h period following 5 days of food deprivation, both Glu/Glu and wild-type (+/+) were given a continuous source of 1 M glucose solution. Although Glu/Glu visited the solution as frequently as +/+, feeding bouts were significantly shorter. These truncated feeding bouts were sufficient to extend Glu/Glu longevity to ca. 80 days, regardless of glucose concentration. When provided continuously, the glucose was completely rejected by Glu/Glu for the first 3 days. However, by day 9, glucose intake levels were similar to those of wild-type cockroaches. The initial feeding lag probably accounted for the lower long-term survival of the Glu/Glu vs the +/+ strain. Under the stress of food deprivation, glucose-averse *B. germanica* modify their feeding behavior toward glucose like many phytophagous insects provided non-host plants in no-choice situations.

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**KEY WORDS:** *Blattella germanica*; German cockroach; feeding behavior; survival; glucose aversion.

### **INTRODUCTION**

Phytophagous insects have evolved various mechanisms for detecting and avoiding consumption of certain allelochemicals (Schoonhoven *et al.*, 1992). These noxious compounds provide little or no nutrition by themselves and may indeed be deleterious, yet they are ingested by insects to varying degrees with edible plant material and processed via mechanisms such as detoxification and diet

<sup>1</sup>The Clorox Company, Technical Center, P.O. Box 493, Pleasanton, California 94566.

<sup>2</sup>To whom correspondence should be addressed. e-mail: Jules.Silverman@Clorox.Com.

mixing (Slansky, 1992). Furthermore, deterrent plants may be ultimately consumed after periods of exposure, particularly after increased food deprivation (Chapman, 1988; Chapman and Bernays, 1989).

Aversion to glucose by the German cockroach, *Blattella germanica*, is a unique example where the nutrient and the deterrent are the same molecule (Silverman and Bieman, 1993). Glucose-averse (*Glu/Glu*) *B. germanica* strains rejected glucose solutions during brief exposure periods (<5 min) after food deprivation intervals not exceeding 2 days. Other than survival in the presence of a very recent selective force, glucose-toxicant mixtures, it is not clear why glucose aversion would have evolved. Glucose-averse and wild-type (+/+) cockroaches survive hemocoelic glucose injections (J.S., unpublished), and the fecundity and longevity of female *Glu/Glu* fed glucose-supplemented diets did not differ from those of +/+ (Silverman, 1995). However, *Glu/Glu* nymphal development and survival were negatively affected by a glucose-supplemented diet: a consequence of reduced diet intake and its impact on growth.

Given that glucose is deterrent and nontoxic, yet negatively impacts some life-history parameters of glucose-averse *B. germanica* by depressing diet consumption (Silverman, 1995), we determined whether *Glu/Glu* cockroaches would accept pure glucose solutions when offered no alternative food source. We also studied how feeding behavior was modified in response to either the intermittent or the continuous presence of this deterrent nutrient and its effect on cockroach survival.

## MATERIALS AND METHODS

Male Orlando Normal (+/+) and T-164 [*Glu/Glu* (Silverman and Bieman, 1993)] *B. germanica* (L.) strains were used in all experiments. Male cockroaches provided a degree of physiological uniformity, without the confounding influences of varying nutritional requirements during nymphal development and female reproduction. Environmental conditions for insect rearing and all experiments were  $27 \pm 1^\circ\text{C}$ ,  $50 \pm 10\%$  RH, and a 12:12 L:D photoperiod.

### Glucose Intake Following Periods of Starvation

Twenty each of +/+ and *Glu/Glu* *B. germanica* (1–21 days old) were placed in each of nine 0.5-L jars. All insects were deprived of food and water for 2 days, then fed 1 M fructose ad lib. to produce a fairly uniform level of satiety. The fructose solution was then replaced with a water vial. On each of 9 successive days the cockroaches from a given jar were assayed for glucose intake, as described by Silverman and Bieman (1993). Briefly, the water vial was replaced with a vial cap containing a solution of 1 M glucose plus 8 mM amaranth dye for 15 min. Individual insects were extracted and assayed spec-

trophotometrically at  $A_{520}$  for the presence of dye. All jars were inspected twice daily to record and discard dead cockroaches that might be cannibalized.

### Behavior of Food-Deprived *B. germanica*

The frequency, duration, and time of feeding events by  $+/+$  and *Glu/Glu* males provided 1 *M* glucose or 1 *M* fructose were determined by videotape analysis. Male cockroaches (10–20 days old, 5 reps/treatment) were housed individually in upright clear plastic rectangular containers (130 × 58 × 58 mm). Sugar solutions were dispensed in 20- $\mu$ l capillary tubes secured by modeling clay. A moistened cotton wick was taped to the inside lid of each container to reduce evaporation of the sugar solutions. Cockroaches were deprived of food, but not water, for 5 days prior to videotaping. Insects were videotaped continuously for 24 h under a 12:12 L:D photocycle with yellow light ( $A_{580}$ ) providing illumination for the camera during the dark phase.

### Longevity of *B. germanica* Provided Glucose as a Sole Food Source

Thirty each of  $+/+$  and *Glu/Glu* cockroaches (1–7 days old) were housed in 45 × 30 × 20-cm plastic containers and provided either water alone or water plus a 0.1, 1.0, or 3.0 *M* glucose solution. Mortality was determined daily and dead insects were removed. In a separate study to determine the effects on longevity of glucose presented at brief intervals,  $+/+$  and *Glu/Glu* (40 1- to 7-day-old males/strain) were provided 1 *M* glucose daily for 15 min. Water was provided continuously and survivorship was determined through 21 days. Glucose intake by surviving cockroaches and glucose-naive *Glu/Glu* was assayed spectrophotometrically, as described above, to determine if *Glu/Glu* became desensitized to the deterrent effects of glucose following brief daily exposure.

### Daily Intake of Glucose

To understand the survival of *Glu/Glu* individuals presented only with glucose over their entire adult life, we monitored daily glucose consumption over an extended period (14 days). Male  $+/+$  and *Glu/Glu* *B. germanica* ( $n = 20$ ) were placed singly in 0.5-L glass jars. The cockroaches were deprived of food for 3 days and water for 2 days, after which 20- $\mu$ l glass capillary tubes, filled to the 13.5- $\mu$ l mark with 1 *M* fructose, were placed with the insects. This procedure ensured that the cockroaches were at about the same level of satiety prior to introducing the glucose solution. The capillary tubes were held upright by a piece of modeling clay. The inside of the jar lid was fashioned with a moistened dental wick to eliminate water loss within the capillary tube, once the lid was secured. The quantity of fructose imbibed was determined 24 h later, after which the tubes were emptied, washed, dried, then filled with 1 *M* glucose.

The volume of glucose imbibed per individual was recorded every 24 h for 14 days. Capillary tubes were refilled as necessary. Three controls were included to determine whether water loss occurred in the absence of feeding.

### **Behavior of Cockroaches Following Extended Glucose Exposure**

Comparable volumes of glucose were imbibed in the above experiment at day 10 by  $+/+$  and *Glu/Glu* males. A videotape analysis was conducted at this time to determine whether the two *B. germanica* strains revealed the same feeding behavior toward glucose following continuous exposure to this sole food source. *Glu/Glu* and  $+/+$  cockroaches (10–20 days old) were housed separately in 0.5-L jars. Glucose (1 M) and water were provided continuously in vial caps. On day 10, individuals ( $n = 5$ ) were housed in upright clear plastic rectangular containers (130 × 58 × 58 mm). Glucose (1 M) was dispensed in 20- $\mu$ l capillary tubes secured by modeling clay and a moistened cotton wick was taped to the inside lid of each container to prevent evaporation of the sugar solution. Insects were videotaped as described earlier.

Sugar consumption and feeding behavior parameters were analyzed using two-way analysis of variance (ANOVA) and one-way ANOVA with Bonferroni simultaneous confidence intervals for mean separation. Weibull analysis was used to compare survivorship distributions (BBN Software Co., 1995).

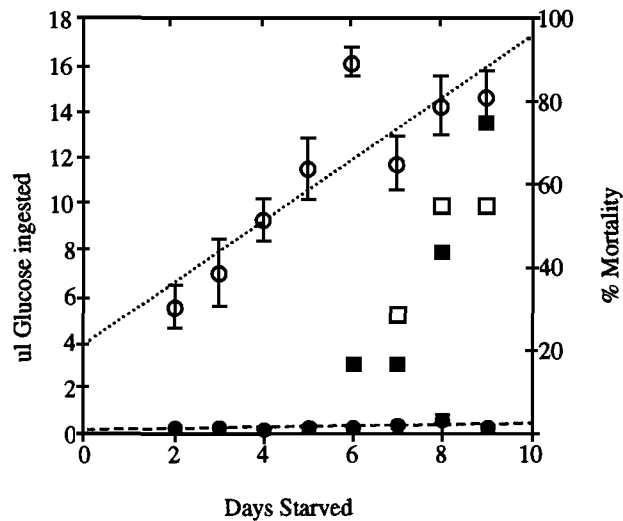
## **RESULTS**

### **Glucose Intake Following Periods of Starvation**

Wild-type males imbibed increasing volumes of 1 M glucose provided for a brief interval as the period of food deprivation increased, to a maximum at 6 days (Fig. 1). In contrast, *Glu/Glu* individuals largely rejected glucose during this brief exposure period, despite up to 9 days of food deprivation. The intensity of this aversion to glucose is dramatic in light of the low survival rate (25%) of *Glu/Glu* individuals deprived of food for 9 days.

### **Behavior of Food-Deprived *B. germanica***

In the 24 h following 5 days of food deprivation, both  $+/+$  and *Glu/Glu* strains visited 1 M fructose and 1 M glucose at the same frequency throughout the observation period (Table I). Least-squares components two-way ANOVA revealed no effect of either strain ( $F = 2.10$ ,  $df = 1,16$ ,  $P > 0.05$ ) or sugar ( $F = 0.01$ ,  $df = 1,16$ ,  $P > 0.05$ ) on feeding frequency or a strain effect ( $F = 3.56$ ,  $df = 1,16$ ,  $P > 0.05$ ) on feeding duration. However, significant sugar ( $F = 6.33$ ,  $df = 1,16$ ,  $P = 0.02$ ) and strain × sugar interaction ( $F = 19.03$ ,



**Fig. 1.** Mean ( $\pm$ SE) intake of 1 M glucose provided daily for 15 min following 1 to 9 days of food deprivation. Wild-type (+/+) glucose intake, open circles; and percentage mortality, open squares. *Glu/Glu* glucose intake, filled circles; and percentage mortality, filled squares.

df = 1,16,  $P = 0.0005$ ) effects were evident, as visits to glucose by *Glu/Glu* individuals were very brief and probably resulted in minimal intake.

#### Longevity of *B. germanica* Provided Glucose as a Sole Food Source

In the presence of glucose solutions as the only food source, *Glu/Glu* males lived approximately one-half as long as +/+ cockroaches. However, they sur-

**Table I.** Frequency and Duration of +/+ and *Glu/Glu* Visits to 1 M Fructose and 1 M Glucose Over a 24-h Interval Following 5 Days of Food Deprivation<sup>a</sup>

| Strain         | Sugar    | Total visits | Mean number of visits $\pm$ SD | Duration (s), mean $\pm$ SD |
|----------------|----------|--------------|--------------------------------|-----------------------------|
| +/+            | Fructose | 131          | 26.2 $\pm$ 12.28a              | 4.4 $\pm$ 1.10a             |
| <i>Glu/Glu</i> | Fructose | 112          | 22.4 $\pm$ 5.68a               | 6.1 $\pm$ 2.11a             |
| +/+            | Glucose  | 152          | 30.4 $\pm$ 11.21a              | 5.7 $\pm$ 1.85a             |
| <i>Glu/Glu</i> | Glucose  | 167          | 33.4 $\pm$ 15.57a              | 1.4 $\pm$ 0.39b             |

<sup>a</sup>Means followed by the same letter are not significantly different ( $P = 0.05$ ; Bonferroni method).

Table II. Longevity of *B. germanica* Males Fed Glucose Solutions

| Glucose conc. (M) | LT-50 (95% CI) days <sup>a</sup> |                |
|-------------------|----------------------------------|----------------|
|                   | +/+                              | <i>Glu/Glu</i> |
| 3.0               | 134.8 (128.3–141.6)              | *              |
| 1.0               | 148.9 (138.1–160.5)              | *              |
| 0.1               | 172.1 (142.0–208.5)              | *              |
| Water only        | 7.8 (7.0–8.7)                    |                |

<sup>a</sup>Rows with asterisks indicate significant difference based on nonoverlapping 95% confidence interval (Weibull method).

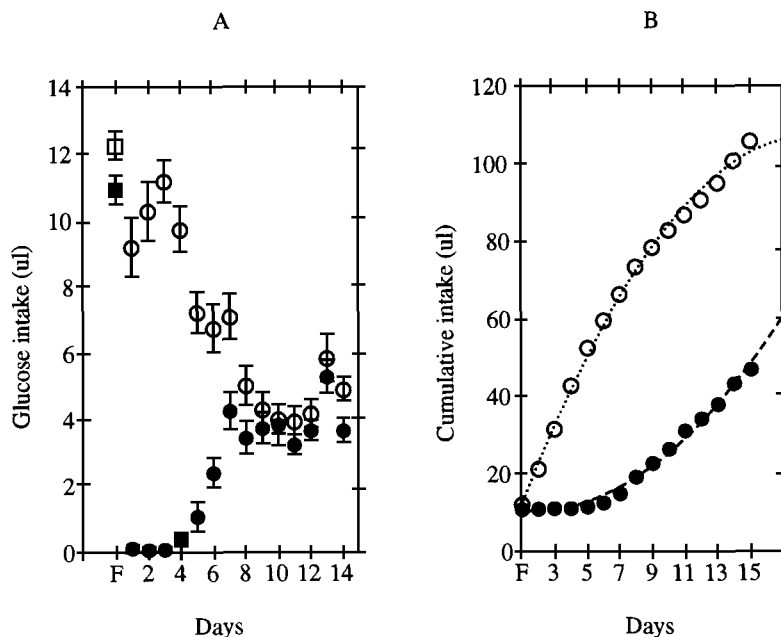
vived roughly 10 times longer than food-deprived insects (Table II). Although it was not evident from the earlier glucose consumption study, *Glu/Glu* individuals must have ingested enough glucose during the short but frequent visits to extend their lives. When 40 males were provided 1 M glucose 15 min daily for 21 days, all but 1 (97.5%) +/+ survived, whereas only 7 (17.5%) *Glu/Glu* survived. This indicates that more frequent encounters with this deterrent were necessary to obtain adequate nutrition. Surviving +/+ and *Glu/Glu* males from the daily 15-min, 21-day exposure study imbibed  $11.1 \pm 0.84$  and  $0.8 \pm 0.62$   $\mu$ l, respectively, of glucose-dye mixture. Naive *Glu/Glu* males imbibed significantly less ( $0.1 \pm 0.01$   $\mu$ l;  $P < 0.05$ ) 1 M glucose than did the *Glu/Glu* survivors, suggesting that some acclimation to glucose may have occurred.

#### Daily Intake of Glucose

After imbibing high levels of 1 M fructose on the day prior to 1 M glucose introduction, daily glucose intake by +/+ males ranged between 9 and 11  $\mu$ l per insect from day 1 to day 4, ca. 7  $\mu$ l from day 5 to day 7, and 4–6  $\mu$ l through 14 days (Fig. 2A). No glucose was imbibed by *Glu/Glu* cockroaches through day 3 despite the absence of alternate food and water. Daily glucose intake by *Glu/Glu* increased to 4.2  $\mu$ l by day 7, then fluctuated between 3.7 and 5  $\mu$ l through day 14. Wild-type males imbibed 2.3-fold more total sugar (day 1 fructose + 14 days of glucose) than *Glu/Glu* (Fig. 2B). *Glu/Glu* imbibed 3.5-fold more sugar than +/+ (23.0 vs 6.5  $\mu$ l) on day 15, when fructose and glucose solutions were provided, demonstrating that surviving *Glu/Glu* (19 of 20) were nutrient deprived despite access to glucose.

#### Behavior of Cockroaches Following Extended Glucose Exposure

Wild-type and *Glu/Glu* cockroaches videotaped over a 24-h period following 10 days of confinement with 1 M glucose as a sole food source imbibed



**Fig. 2.** Mean ( $\pm$ SE) intake of 1 M glucose provided continuously for 14 days. F = 1 M fructose provided on day 1. Wild-type (+/+), open circles; *Glu/Glu*, filled circles. (A) Glucose (fructose) intake per day. (B) Cumulative sugar intake.

similar quantities of glucose ( $3.2 \pm 1.48$  and  $3.6 \pm 0.57 \mu\text{l}$ , respectively;  $P = 0.59$ ,  $t$  test). These amounts were comparable to the levels imbibed in the prior experiment. Differences in feeding duration between *Glu/Glu* ( $1.4 \pm 0.16$  s/visit) and +/+ ( $2.3 \pm 1.14$  s/visit) could not be separated statistically ( $P = 0.06$ ,  $t$  test). Also, the number of visits per insect to the glucose source by *Glu/Glu* ( $15.2 \pm 5.89$  visits/insect) and +/+ ( $9.4 \pm 5.86$  visits/insect) were not different ( $P = 0.08$ ,  $t$  test).

## DISCUSSION

As expected, food-deprived +/+ males offered glucose for a brief period imbibed more glucose with increased starvation to a maximum of  $16 \mu\text{l}$ , which may be close to proventricular capacity. *Glu/Glu* males imbibed, at most, levels of glucose barely within the range of detection, despite prolonged starvation, leading to the death of most of the insects. When exposed for 15 min each day, the few visits by *Glu/Glu* males to glucose provided inadequate nutrition, resulting in a high mortality. Although the general feeding behavior of *Glu/Glu* and

wild-type insects was similar, evidenced by their response to fructose, visits to glucose by *Glu/Glu* were very brief, resulting in a limited intake. Surprisingly, *Glu/Glu*'s truncated feeding bouts on glucose were not balanced by increased visits. Silverman (1986) determined that male (+/+) visited a food source about 10 times during a 96-h period and fed for 5–6 min per visit. In contrast, +/+, in the current study, visited the food source far more frequently and for shorter periods of time. Silverman (1986) employed an arena configuration with a considerably greater harborage-to-resource distance than used in the current study, which served to decrease the number of food encounters, and a solid food source, which probably prolonged nutrient handling and processing. Also, the frequent encounters with 1 M glucose may have been related to water as well as nutritional needs.

Male *Glu/Glu* provided glucose solutions ad libitum survived ca. 10× longer than food-deprived *B. germanica*, yet shorter than similarly provisioned +/+ males. Minimal sugar intake during brief feeding bouts without compensation from increased glucose visitation probably dropped the insect's nutritional status below the level required for homeostasis. However, Silverman (1995) reported survival rates of male *Glu/Glu* fed complex diets with or without glucose that were similar to those recorded here. Therefore, decreased *Glu/Glu* longevity cannot be explained solely in terms of glucose ingestion, as *Glu/Glu* males are apparently inherently short-lived. Ye *et al.* (1994) determined that survival time during food deprivation in *Drosophila melanogaster* was heritable; however, strain longevity data for fully fed flies were not reported.

Although male *Glu/Glu* survived for as long as 80 days, we did not measure their reproductive fitness, which may have been compromised by nutritional stress. Silverman and Bieman (1993) reported that glucose aversion was concentration dependent. In light of this, we would have expected greater *Glu/Glu* longevity with a decreased glucose concentration. Similar longevity of males provided glucose solutions from 0.1 to 3.0 M suggests that a behavioral response threshold was exceeded at the lowest concentration. Finally, the long-term survival of males of both strains fed only glucose was surprising. Male lipid and protein requirements may be minimal and provided from nymphal reserves. In addition, amino acid synthesis from glucose by microbial symbiotes may occur as in *Periplaneta americana* (Lipke *et al.*, 1965).

Since food-deprived glucose-naive *Glu/Glu* largely rejected glucose provided for 15 min, yet cockroaches provided glucose ad lib. survived ca. 80 days, *Glu/Glu* may have modified its behavioral response to this deterrent nutrient in the face of starvation. Although glucose intake by *Glu/Glu* which survived 15-min/21-day glucose presentations was minimal, it was significantly greater than that of glucose-naive *Glu/Glu*. Also, records of *Glu/Glu* daily glucose intake provide some explanation for this strains' prolonged survival through desensitization, where following a period of complete rejection, consumption levels

were similar to those of +/+ . Ultimate acceptance of glucose by *Glu/Glu* by day 4 might be explained by changes in sensory perception modulated by hunger, although it is difficult to distinguish desensitization from the effects of deprivation on behavior (Papaj and Prokopy, 1989). Response decrement mechanisms such as sensory adaptation and/or effector fatigue may play some role in glucose acceptance by *Glu/Glu* but can be ruled out as the sole causes of waning due to the extended period of time between stimulus applications in one study, which is more consistent with habituation (Hinde, 1970). In many instances, increased food deprivation by herbivores increases the acceptance of nonhost plants (Chapman and Bernays, 1989; Grabstein and Scriber, 1982; Schoonhoven and Meerman, 1978), with gustatory habituation following a relatively long preexposure to the deterrent allelochemical (Glendinning and Gonzalez, 1995). Tolerance of deterrents by food-deprived insects may not be true habituation but, rather, an overriding of the deterrent response by the increased state of deprivation, which, by increasing contact with the deterrent, may be an important component of the habituation process (Szentisi and Bernays, 1984). Although deprivation is most likely involved in *Glu/Glu*'s decision to imbibe glucose, there is also evidence for habituation to deterrents in satiated insects (Usher *et al.*, 1988; Szentisi and Bernays, 1984). It is noteworthy that the duration of *Glu/Glu* and +/+ feeding bouts could be distinguished in the study using 5-day food-deprived glucose-naive insects, but not when 10-day glucose-preexposed insects were observed. Longer +/+ feeding bouts (5.7 s) following prolonged starvation in the former study accounted for the strain difference. After +/+ corrected the immediate nutrient deficit, they followed a feeding profile similar to that of *Glu/Glu*. These initial feeding bouts by +/+ resulted in a much greater cumulative glucose intake, which probably accounted for its survival advantage.

Efforts to understand sensory and central mechanisms responsible for deterrent allelochemical rejection and desensitization, including habituation, in phytophagous arthropods can be complicated by deleterious postingestion effects. Glucose as a deterrent nutrient to *Glu/Glu* may provide a model for studying preingestive processes without inducing detoxification mechanisms.

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